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Leaf Flavonoids and Pericarp Anthocyanins from *Clintonia udensis* (Liliaceae)

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As chemotaxonomical markers, the flavonoid components in the leaves of *Clintonia udensis* (Liliaceae) were surveyed. Five flavonol glycosides were isolated by column and paper chromatography, and four except one, which was minor, were identified as follows by PC and HPLC in comparisons with authentic specimens, UV spectra and acid hydrolysis: quercetin 3-*O*-diglucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside and kaempferol 3,7-di-*O*-glucoside. The pericarp anthocyanins of *C. udensis* var. *udensis*, which bears blue pericarp, and f. *nigra* (black pericarp) were also investigated to differentiate them. Delphinidin 3-*O*-rutinoside and cyanidin 3-*O*-rutinoside were found in the pericarps of the two taxa, and it was proved that the difference of the colors was due to the difference of quantitative ratio of their anthocyanins.

Introduction

The genus *Clintonia* (Liliaceae) consists of several species and is disjunctively distributed in North America and East Asia (Willis 1966). Among their species, only *C. udensis* Trautv. et Mey. is native to East Asia including Japan. The cytological studies of the genus have been performed by some workers (Matsuura and Suto 1935, Sato 1942, Hara and Kurosawa 1963, Kurosawa 1966, Lee 1969, Kapoor 1970, Nakamura and Taira 1987), but its chemical ingredients have not fully been investigated. Only one report so far published has deal with the detection of cyanidin 3-*O*-rutinoside and delphinidin 3-*O*-rutinoside from the blue pericarps of *C. udensis* (Yoshitama and Ishikura 1988). Williams (1975) failed to detect the flavonoids in the leaves of other two *Clintonia* species, *C. andrewsiana* Torrey and *C. borealis* Rafin.

In this paper, we describe the flavonoid composition in the leaves of *C. udensis* which was most widely distributed in the genus, native to East Asia and occasionally subdivided into some varieties and forms, var. *udensis*, var. *lanceolata* Hayashi, f. *leucocarpa* Hayashi (Hayashi 1954) and f. *nigra* Takeda (Mizushima 1960). Moreover, qualitative and quantitative comparisons of the anthocyanins were performed between the two taxa, var. *udensis* (pericarp, blue) and f. *nigra* (black), which were distinguishable by pericarp colors.

Materials and Methods

Plant materials *Clintonia udensis* was collected in the following four places: Yashajin Pass, Yamanashi Pref.; Mt. Fuji, Yamanashi Pref.; Mt. Nekomagatake, Fukushima Pref.; and Suzuran Pass, Nagano Pref., in the flowering and the fruiting time in 1992. The voucher specimens were deposited in the Showa College of Pharmaceutical Sciences.

Isolation and identification of leaf flavonoids The lyophilized leaves were extracted with 80% methanol and concentrated in vacuo. The concentrates were washed several times with petroleum ether and then extracted with ethyl acetate. Organic layer in which flavonoids were mostly contained was concentrated to dryness, dissolved with water and chromatographed on a polyamide column with the following solvent system: methanol-water, gradient from 0% to 100% methanol by 10%. Eluents were isolated by preparative paper chromatography (PPC) and finally purified by Sephadex LH-20 column (solvent system: 70% methanol). The flavonoids were identified by HPLC and TLC in comparisons with authentic specimens and UV spectral survey, and characterization of aglycones and sugars which was obtained by acid hydrolysis.

Fresh pericarps were extracted with MAW (MeOH-AcOH-H_2O = 20:15:65) and concentrated to a small volume. The crops were chromatographed on a Sephadex LH-20 column (solvent system: MAW) and then subjected to paper chromatography (solvent system: n-BuOH-AcOH-H_2O = 4:1:5, upper phase). Purified anthocyanins were identified by HPLC, PC and acid hydrolysis in general way (Hayashi et al. 1989).

Acid hydrolysis Glycosides were hydrolyzed with 10% HCl for 0.5 hr at 100°C. The hydrolysates (aglycones and sugars) were identified as usual (Hayashi et al. 1989) by HPLC and PC.

UV spectra UV spectra were measured in methanol solution in addition to usual shift reagents (see, Table 2) according to Mabry et al. (1970).

HPLC survey HPLC separations of flavonol aglycones, anthocyanins and anthocyanidins were performed with Shim-pak CLC-ODS (I.D., 0.15mm × 6.0cm) column, Shimadzu SPD-M6A detector, at flow-rate: 1.0 ml/min, detection: 275 nm (flavonols) or 530 nm (anthocyanins and anthocyanidins) according to Hayashi et al. (1989). 80% MeOH (for flavonol aglycones) and MeOH-AcOH-H_2O (20:15:65) (for anthocyanins and anthocyanidins) were used as eluents.

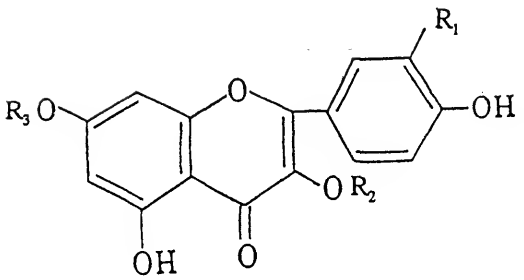
Results

Leaf flavonoids Four (**1–4**) of the five flavonoids were isolated, were obtained in crystals or in pure solutions and identified as described below.

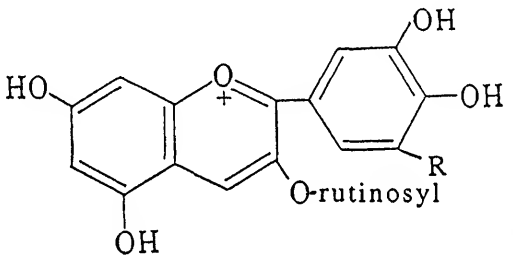
UV spectra of **1** showed the presence of free 5-, 7-, 3'- and 4'-hydroxyls and a substituted 3-hydroxyl group (Table 1). An aglycone obtained by acid hydrolysis was identified as quercetin by HPLC comparison of retention time (Rt) and absorption maxima with authentic specimen (Table 3). Rf values of glycosidic sugar coincided with that of authentic glucose on the chromatogram (Table 3). Moreover, it was proved by Rf, especially with 15% AcOH, of TLC that flavonoid **1** was diglycoside (Table 1). Thus, flavonoid **1** was identified as quercetin 3-*O*-diglucoside (Fig. 1).

UV spectra of **2** showed the presence of free 5- and 4'-hydroxyl and substituted 3- and 7-hydroxyl groups (Table 2). An aglycone and a sugar, which were given by acid hydrolysis, were identified as kaempferol and glucose, respectively, by HPLC and PC comparisons with authentic samples (Table 3). From the results described above, flavonoid **2** was identified as kaempferol 3,7-di-*O*-glucoside (Fig. 1).

Acid hydrolysis of flavonoid **3** gave kaempferol and glucose, which were identified by HPLC and PC comparison with authentic specimens (Table 3). It was shown by UV spectra that glucosyl moiety was



R_1 = OH, R_2 = diglucosyl, R_3 = H : quercetin 3-diglucoside (1)
R_1 = H, R_2 = R_3 = glucosyl : kaempferol 3,7-diglucoside (2)
R_1 =H, R_2 = glucosyl, R_3 = H: kaempferol 3-glucoside (astragalin, 3)
R_1 = H, R_2 =rutinosyl, R_3 = H : kaempferol 3-rutinoside (nicotiflorin, 4)



R = OH : delphinidin 3-rutinoside (5)
R = H : cyanidin 3-rutinoside (keracyanin, 6)

Fig. 1. The structures of flavonols and anthocyanins in *Clintonia udensis*.

attached to 3-hydroxyl of kaempferol (Table 2). Accordingly, flavonoid **3** was regarded as kaempferol 3-*O*-glucoside (astragalin, Fig. 1).

UV spectra of flavonoid **4** indicated the presence of free 5-, 7- and 4'-hydroxyl and a substituted 3-hydroxyl group (Table 2). An aglycone and two sugars were obtained by acid hydrolysis and identified as kaempferol, glucose and rhamnose, respectively, by HPLC and PC behaviors (Table 3). Finally, PC and HPLC data of flavonoid **4** completely agreed with authentic kaempferol 3-*O*-rutinoside (nicotiflorin, Fig. 1; Table 1).

Pericarp anthocyanins From the blue pericarps of var. *udensis*, two anthocyanins were isolated. It was proved that they were delphinidin 3-*O*-rutinoside (**5**) and cyanidin 3-*O*-rutinoside (keracyanin, **6**) by HPLC and PC qualification of anthocyanidins and sugars, which were liberated by acid hydrolysis, and by direct HPLC comparison of original anthocyanins with authentic specimens (Table 4).

In parallel, qualitative and quantitative HPLC survey was performed on the blue (var. *udensis*) and black (f. *nigra*) pericarps. As these results, their anthocyanins were qualitatively identical. However, it was proved that the content of cyanidin 3-*O*-rutinoside was lower than that of delphinidin 3-*O*-rutinoside in the cases of both specimens, but the content of the former anthocyanin in black pericarp

Table 1. Chromatographic data of flavonol glycosides in the leaves of *Clintonia udensis*

Flavonols	Rf values		Colors	
	BAW (PC)	15% AcOH (TLC)*	UV	UV/NH_3
1	0.44	0.61	purple	yellow
2	0.61	0.71	purple	dark greenish yellow
3	0.78	0.43	purple	dark greenish yellow
4	0.85	–	purple	dark greenish yellow
authentic sample: kaempferol 3-rutinoside	–	0.71	purple	dark greenish yellow

BAW = n-BuOH-AcOH-H_2O (4:1:5, upper phase). **1** = quercetin 3-diglucoside, **2** = kaempferol 3,7-diglucoside, **3** = kaempferol 3-glucoside and **4** = kaempferol 3-rutinoside.
* Abisel SF cellulose plate.

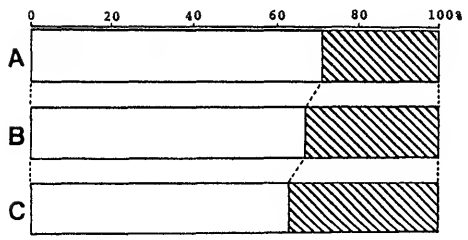


Fig. 2. Comparison of anthocyanin contents, delphinidin 3-rutinoside (□) and cyanidin 3-rutinoside (▨), in blue (var. *udensis*, A), intermediate (B) and black (f. *nigra*, C) pericarps of *Clintonia udensis*.

was relatively higher than that of blue one (Fig. 2).

Discussion

The flavonoids which were found in the leaves of *Clintonia udensis* in this experiment were the glycosides of quercetin and kaempferol. On the other hand, these flavonol glycosides have not been detected from *C. andrewsiana* and *C. borealis* in North America although flavonols occur most frequently in the family Liliaceae (Williams, 1975). Williams reported that quercetin and kaempferol were detected in 40% and 42% in the leaf survey of 168 species of the family. Though we surveyed only four populations of *C. udensis* in Japan, their flavonoid compositions were, at least, qualitatively uniform. The fact seems to show that the occurrence of quercetin 3-*O*-diglucoside, kaempferol 3-*O*-glucoside, kaempferol 3-*O*-rutinoside and kaempferol 3,7-*di*-*O*-glucoside is the diagnostic chemotaxonomic feature of *C. udensis*, but a few North American species remain unanalyzed.

As anthocyanins in the blue pericarps of *C. udensis* var. *udensis*, delphinidin 3-*O*-rutinoside and cyanidin 3-*O*-rutinoside have been found by Yoshitama and Ishikura (1988), which were also confirmed in this experiment. We also surveyed about the anthocyanin components in black pericarps of *C. udensis* f. *nigra* which was distinguished from var. *udensis* by their pericarps colors (Mizushima 1960). The quality of the anthocyanins was the same, while only the ratio of the two anthocyanin contents between two taxa was different. Moreover, intermediate pericarp color in two taxa has been recognized by Mizushima (1960). It was shown that its anthocyanin composition was also

Table 2. UV spectral data of flavonol glycosides in the leaves of *Clintonia udensis*

Flavonols	λ max (nm)					
	in MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3BO_3
1	252	268	270	267	265	265sh
	344	341	417	360	341	341
		423		398	417	382
		(inc)				
2	267	273	274	274	264	266
	341	387	341	341	341	305
		(inc)	380	383	381	342
3	267	275	268	275	275	267
	341	326	300	302	306	341
		341	341	341	381	
		400	381	381		
		(inc)				
4	267	276	280	275	274	268
	341	330sh	304	303	309	313
		341sh	341	341	380	341
		381	380	380		
		(inc)				

inc = remarkable increase in intensity relative to the methanol spectrum. sh = shoulder.

Table 3. Rt (retention times) of aglycones and Rf values of sugars by acid hydrolysis from flavonol glycosides in the leaves of *Clintonia udensis*

Compounds	Aglycones*	Sugars**
	Rt (min)	Rf values
1	3.67	0.37
2	4.12	0.38
3	4.13	0.37
4	4.12	0.37, 0.63
authentic specimens		
quercetin	3.59	–
kaempferol	4.14	–
glucose	–	0.39
rhamnose	–	0.59

*HPLC: Eluent, 80% MeOH; flow-rate, 1.0ml/min; detection, 275nm; column Shim-pack CLC-ODS 0.15mm×6.0cm.
**PC: solvent system, BAW = n-BuOH-AcOH-H_2O (4:1:2, upper phase).

Table 4. Rt (retention times) and Rf values of anthocyanins, and their hydrolysates (anthocyanidins and sugars) in the pericarps of *Clintonia udensis*

Compounds	Anthocyanins	Anthocyanidins	Sugars
	Rt (min)*	Rt (min)*	Rf values**
5	5.63	4.37	0.25, 0.41
6	6.96	6.17	0.25, 0.40
authentic specimens:			
delphinidin 3-rutinoside	5.74	–	–
cyanidin 3-rutinoside	6.82	–	–
delphinidin	–	4.44	–
cyanidin	–	6.18	–
glucose	–	–	0.26
rhamnose	–	–	0.41

*HPLC: Eluents; MAW = MeOH-AcOH-H2O (20:15:65), flow-rate; 1.0ml/min. Detection: 530nm.
**PC: solvent system; BAW = n-BuOH-AcOH-H_2O (4:1:5, upper phase).

qualitatively identical with those of two taxa, and their contents continuously varied from blue to black via intermediate color (Fig. 2). Thus, two taxa, var. *udensis* and f. *nigra* are not chemically distinguishable.

In this preparatory experiment, we surveyed only *C. udensis* which was only distributed in East Asia in the genus *Clintonia*. Its flavonoids seem to be different from those of two species from North America. Further survey of other *Clintonia* species are now in progress.

References

Hara H. and Kurosawa S. 1963. Cytotaxonomical studies on Japono-Himalayan elements (1). J. Jpn. Bot. **38**: 7–10 (in Japanese with English summary).

Hayashi K., Ootani S. and Iwashina T. 1989. Comparative analysis of the flavonoid and related compounds occurring in three varieties of *Iris setosa* in the flora of Japan. Sci. Rep. Res. Inst. Evolut. Biol. **6**: 30–60 (in Japanese with English summary).

Hayashi Y. 1954. New variety formas of herbaceous plants. J. Jpn. Bot. **29**: 199–200 (in Japanese).

Kapoor B. M. 1970. In IOPB chromosome number reports XXVII. Taxon **19**: 437–442.

Kurosawa S. 1966. Cytological studies on some Eastern Himalayan Plants. *In* Hara H. (ed.), Fl. East. Himal., Univ. of Tokyo Press: 658–670.

Lee Y. N. 1969. Chromosome number of flowering plants in Korea (2). J. Korea Res. Inst. Better Living **5**: 127–129.

Mabry T. J., Markham K. R. and Thomas M. B. 1970. The Systematic Identification of Flavonoids. Springer-Verlag, London.

Matsuura H. and Suto T. 1935. Contributions of the idiogram study in phanerogamous plants I. J. Fac. Sci. Hokkaido Imp. Univ., Ser. **5**: 70–71.

Mizushima M. 1960. A note on the colour of "berry" of *Clintonia alpina* var. *udensis*. J. Jpn. Bot. **35**: 179 (in Japanese with English summary).

Nakamura T. and Taira M. 1987. Cytological studies in family Liliaceae of Japan IV. Chromosome number of *Clintonia*. La Kromosomo II-45: 1433–1439 (in Japanese with English summary).

Sato D. 1942. Karyotype alteration and phylogeny in Liliaceae and allied families. Jap. Jour. Bot. **12**: 57–161.

Williams C. A. 1975. Biosystematics of the monocotyledoneae – Flavonoid patterns in leaves of the Liliaceae –. Biochem. Syst. Ecol. **3**: 229–234.

Willis J. C. 1966. A dictionary of the flowering plants and ferns. Cambridge at the University Press, Delhi-India (Seventh Ed.) 262.

Yoshitama K. and Ishikura N. 1988. The distribution of anthocyanin pigments in the flora of Japan. *In*: Hayashi K. (ed.) Plant Pigments – An introduction to Research and Experiments –. Enlarged edition. Yokendo, Tokyo. p.501 (in Japanese).

中村卓造，大塚真紀，中川裕二，金田典子，船本常男，富田香織，岩科 司：**ツバメオモトの葉に含まれるフラボノイド成分と果皮のアントシアニン**

ツバメオモト（***Clintonia udensis*** Trautv. et Mey.）の葉のフラボノイド成分がこの種の化学分類学的指標として用いることができるかどうかを検討するために，フラボノイド成分の分離同定を行った．5種類のフラボノール配糖体がカラムおよび濾紙クロマトグラフィーによって分離され，そのうち4種類が基準標品との各種クロマト法による比較，UV吸収スペクトルおよび加水分分解産物の定性等により，それぞれquercetin 3-*O*-diglucoside, kaempferol 3, 7-*O*-diglucoside, kaempferol 3-*O*-glucoside および kaempferol 3-*O*-rutinoside と同定された．これまでに，ツバメオモト属では，北米産の *C. andrewsiana* および *C. borealis* についてフラボノイドの分析が行われているが（Williams 1975），これらの種からは quercetin あるいは kaempferol の配糖体は報告されていないことから，今回明らかにしたフラボノイド組成はこの属の中でツバメオモトだけに固有なものと推定された．これらと平行して，ツバメオモトの果皮に含まれるアントシアニンについても分離同定を行った．delphinidin 3-*O*-rutinoside と cyanidin 3-*O*-rutinoside が検出され，以前の報告（吉玉，石倉1988）と一致したが，従来果皮の色でのみ区別されていた var. ***udensis***（青色）と f. ***nigra***（黒色）の定量および定性実験では，この両者に含まれる2種類のアントシアニンは質的にまったく同一で，色素の相対量によってのみ異なることが判明し，化学分類学的にはこれらの変種および品種は広義の var. ***udensis*** に包含されるべきと推定された．